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Request for grant of a Patent

Form 1/77 **Patents Act 1977**

❶ Title of invention

1 Please give the title of the invention: THERAPEUTIC ANTIBODY COMPOSITION

❷ Applicant's details

☐ **First or only applicant**

2a If you are applying as a corporate body please give:
Corporate Name: SmithKline Beecham Biologicals s.a.

Country *(and State of incorporation, if appropriate)*: Beligan

2b If you are applying as an individual or one of a partnership please give in full:

Surname

Forenames

2c **In all cases,** please given the following details:

Address: rue de l'Institut 89, B-1330 Rixensart

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Country: Belgium

ADP number *(if known)*: **5800974002** ✓ S7911700Ins

2d, 2e and 2f: If there are further applicants please provide details on a separate sheet of paper

☐ **Second applicant *(if any)***

2d If you are applying as a corporate body please give:
Corporate Name
Peptide Therapeutics Limited

Country *(and State of Incorporation, if appropriate)*

2e If you are applying as an individual or one of a partnership please give in full:

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Forenames:

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③ **Address for service details**

3a Have you appointed an agent to deal with your application?
Yes ☒ No ☐ ⇒ ***go to 3b***

④

please give details below

Agent's name K L PRIVETT

Agent's address SmithKline Beecham
Corporate Intellectual Property
New Horizons Court
Great West Road
Brentford, Middlesex

Postcode TW8 9EP
Agent's ADP number 58009776002

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3b If you have not appointed an agent please give a name and address in the United Kingdom to which all correspondence will be sent:

Name

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❹ Reference number

4. Agent's or applicant's reference number *(if applicable)* B45207

❺ Claiming an earlier application date

5. Are you claiming that this application be treated as having been filed on the date of filing of an earlier application?

Please mark correct box

Yes ☐ No ☒ ➲ *go to 6*

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☐ filing date *(day month year)*

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Please give the date in all number format, for example, 31/05/90 for 31 May 1990

Country of Filing	Priority application number *(if known)*	Filing Date *(day, month, year)*

⑦ Inventorship

⑦ any applicant is not an inventor
◦ there is an inventor who is not
◦ an applicant, or
◦ any applicant is a corporate body.

7. Are you (the applicant or applicants) the sole inventor or the joint inventors?
Please mark correct box

Yes ☐ No ☒ ⇒ ***A Statement of Inventorship on Patents form 7/77 will need to be filed (see Rule 15).).***

⑧ Checklist

⑧ Please supply duplicates of claim(s), abstract, description and drawings).

8a Please fill in the number of sheets for each of the following types of document contained in this application

Continuation sheets for this Patents Form 1/77 —

Claim(s) 4 Description 28

Abstract — Drawing(s) 17

8b Which of the following documents also accompanies the application?

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Patents Form 7/77 - Statement of Inventorship and Right to Grant —

Patents Form 9/77 - Preliminary Examination Report —

Patents Form 10/77 - Request for Substantive Examination —

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K L PRIVETT
Chartered Patent Attorney
Attorney for the Applicant

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B45207

Therapeutic Antibody Composition and Use

The present invention relates to novel therapeutic compositions containing an anti-IgE antibody, and its use in the preparation of medicaments for the treatment or prophylaxis of IgE-mediated allergies. Where legally permissible, the invention also provides a method of treatment/prophylaxis using such compositions of antibodies.

In a second aspect, the invention relates to use of the antibody for determination of therapeutically "useful" sequences within the IgE molecule and mimotopes thereof, which may be used to form the basis of an anti-IgE vaccine for treatment/prophylaxis of allergic disease.

The term "antibody" herein is used to refer to a molecule having a useful antigen binding specificity. Those skilled in the art will readily appreciate that this term may also cover polypeptides which are fragments of or derivatives of antibodies yet which can show the same or a closely similar functionality. Such antibody fragments or derivatives are intended to be encompassed by the term antibody as used herein.

Various classes of antibodies are known which may generically be called "anti-IgE antibodies". These may recognise one or more regions of the IgE molecule. For example, monoclonal antibodies are known which bind to the human IgE heavy chain binding site for mast cells (WO 93/04173). More specifically, antibodies are known which bind to the IgE Fc domain (WO 89/04834 and WO 90/15878).

Depending on their particular nature, anti-IgE antibodies may bind IgE either in its receptor-bound state or non-receptor-bound (i.e. free solution or bound to a solid phase).

Known Anti-IgE antibodies are generally anaphylactogenic, i.e. they cause triggering of basophils or mast cells by cross-linking of IgE bound to its high affinity receptor (FcεRI), resulting in degranulation and release of histamine. Consequently such

antibodies are therapeutically useless and indeed are likely to be positively dangerous if administered to a patient.

Nonetheless, such antibodies are commercially available and have been sold as IgE detection agents for use in techniques such as Western blotting and immunohistochemistry. Those skilled in the art fully understand that in this field the mere existence of anti-IgE activity in an antibody does not imply any useful therapeutic or prophylactic properties.

Much work has been carried out by those skilled in the art to identify specific anti-IgE antibodies which do have some beneficial effects against IgE-mediated allergic reaction (WO 90/15878, WO 89/04834, WO 93/05810). Attempts have also been made to identify epitopes recognised by useful antibodies, to create peptide mimetics of such epitopes and to use those as immunogens to produce anti-IgE antibodies. Based on the present state of knowledge in this area, and despite enormous scientific interest and endeavour, there is little or no predictability of what characteristics any antibody or epitope may have and whether or not it might have a positive or negative clinical effect on a patient.

According to the present invention, it has been found that an anti-IgE antibody, designated PTmAb0011 (In-house monoclonal antibody), has the following properties:

(1) It is capable of binding human IgE in its non-receptor-bound state (i.e. in free solution or when bound to a solid phase support e.g. an ELISA plate).

(2) It is capable of binding IgE bound to its high affinity receptor (FcεRI).

(3) It will prevent binding of IgE to the high affinity IgE receptor (FcεRI).

(4) It will not prevent IgE binding to the low affinity IgE receptor (FcεRII).

(5) It inhibits degranulation of human blood basophils following exposure to antigen.

This antibody is potentially useful as a therapeutic/prophylactic agent due to its combination of non-anaphylactogenicity and ability to stabilise basophils.

According to the invention the antibody may be used in the manufacture of a medicament for the treatment/prophylaxis of IgE-mediated immune response such as allergy.

In addition, the antibody can be used to determine sequences within the IgE molecule and mimotopes thereof, which could form the basis of an anti-IgE vaccine, by techniques familiar in the art including but not limited to, the use of phage display (WO 92/07077).

The antibody may be used in a method of treatment of IgE-mediated immune response such as allergy by administering antibody, fragments thereof, or humanised versions of, to a patient as a vaccination to provide passive immune protection against the adverse effects of exposure of the patient to allergen.

The antibody may be used according to the invention against all types of IgE-mediated allergies since the antibody is not dependent on the antigen specificity of the IgE but will react with the total IgE pool of the patient.

In view of the foregoing description it will now be clear to those skilled in the art that the above-mentioned antibody could provide a useful therapeutic/prophylactic agent. This agent could be administered (e.g. as a vaccination) by routine clinically acceptable means in appropriate dosage forms and dosage regimes to provide patients with relief from or protection against the adverse clinical effects and symptoms of immune reaction to antigen, e.g. in treatment/prophylaxis of allergy.

Similarly, epitopes from the IgE molecule or mimotopes thereof, which have been defined by use of this antibody, may also be used in an active vaccination approach to the treatment of allergy.

Polypeptides have been identified that are recognised by the anti IgE antibody and which when conjugated to a suitable immunogenic carrier, and administered to a host animal, will induce the raising of antibodies to said polypeptide which will have anti IgE properties.

The following polypeptides, although not limiting, specifically exemplify this aspect of the invention

HCQQVFFPQDYLWCQRG – Seq ID No 1
SCREVWLGGSEMIMDCE – Seq ID No 2
ECNQNLSGSLRHVDLNC – Seq ID No 3
DCEEPMCSPVLLQKLKP – Seq ID No 4
SCREVWLGGSEMIMDCE – Seq ID No 5
RCDQQLPRDSYTFCMMS – Seq ID No 6
SCPAFPREGDLCAPPTV – Seq ID No 7
FCPEPICSPPLSRMTLS – Seq ID No 8
VCDECVSRELAL – Seq ID No 9
WCLEPECAPGLL – Seq ID No 10
VCDECVSRELAL – Seq ID No 11
DCLSKGQMADLC – Seq ID No 12
SCQGREVRRECW – Seq ID No 13
WCREVWLGESETIMDCE – Seq ID 14
ACREVWLGESETIMDCD – Seq ID 15
GCAEPKCWQALHQKLKP – Seq ID 16

The invention is illustrated by the results shown by data in the following figures, in which:

Figure 1 shows the concentration dependent binding of antibody PTmAb0011 to IgE.

Figure 2 shows the concentration dependent inhibition of IgE binding to an FcεR1α/IgG construct with antibody PTmAb0011 compared to control.

Figure 3 shows the concentration dependent inhibition of IgE binding to clipped ectodomain of FcεRIα-bound directly to plastic plates, by antibody PTmAb0011, compared to control.

Figure 4 shows the lack of inhibition of IgE binding to FcεRII (CD23) by antibody PTmAb0011.

Figure 5 shows the concentration-dependent blocking of histamine release from allergic human blood basophils with antibody PTmAb0011 compared to control.

Antibody Specification

PTmAb0011 is a mouse IgG1 monoclonal antibody deposited as Budapest Treaty patent deposit at ECACC on 8th March 1999 under Accession No. 99030805.

Assays and Test Methods

IgE binding assay.

1.

2. Materials

Material	**Source (Product Code)**
96 well tissue EIA/RIA plates	Costar (3590)

Reservoir trays	Costar (4870)
Phosphate-buffered saline tablets	Sigma (P-4417)
Bovine serum albumin	Sigma (A-2153)
Tween 20	Sigma (P-1379)
o-Phenylenediamine	Sigma (P-8287)
25% v/v sulphuric acid	Not critical
Phosphate-citrate buffer with sodium perborate	Sigma (P-4922)
Human/mouse chimeric IgE	Serotec (MCA333B)
Mouse anti-human IgE mAb (Clone 0277)	Biogenesis (5118-5004)
Sheep anti-mouse IgG-HRP	Serotec (AACO1P)
Sodium carbonate (Analar grade)	BDH (102404H)
Sodium hydrogen carbonate (Analar grade)	BDH (102474V)

3.

4. Equipment

Equipment	**Supplier**
Multichannel pipette	Finnpipette or equivalent
MRX ELISA plate reader	Dynex Technologies
DELFIA 1296-026 Platewasher	Wallac

5.

6. Method

ELISA protocol for the detection of mouse anti-human IgE mAbs.

6.1 Coat plates overnight at 4°C with 100 µl/well human IgE diluted to 1 µg/ml in carbonate buffer (1.59 g sodium carbonate and 2.93 g sodium hydrogen carbonate dissolved to 1 litre after adjustment to pH 9.6).

6.2 Wash wells three times with 700 µl/wash (PBS/Tween 0.05%).

6.3 Block wells with 150 µl of PBS/Tween 0.05%/50 g/l BSA for 2 hours at 37°C.

6.4 Add 100 µl/well of anti-human IgE antibody (standard) over the concentration range of $2x10^6$ to 25.6 pg/ml or test supernatant added undiluted or over a range of dilutions, typically up to 1/100, with dilutions prepared in PBS/Tween 0.05%/10 g/l BSA. Incubate the plate for 1 hour at 37°C.

6.5 Wash plates as described.

6.6 Add 100 µl/well sheep anti-mouse IgG-HRP conjugated antibody diluted to 1/4000 in PBS/0.05% Tween/10 g/l BSA and incubate for 1 hour at 37°C.

6.7 Wash plates as described.

6.8 Add 100 µl/well OPD substrate and incubate at room temperature in the dark for 10-20min. Stop the reaction by the addition of 50 µl/well 25% v/v sulphuric acid and read the O.D. at 490nm.

7. Treatment of Results

A standard curve of known concentrations of mouse anti-human IgE antibody *vs* O.D. is constructed. Test supernatants will be considered positive for antibody if their O.D. value is greater than the mean of background plus three times the standard deviation of the background (mean ±3SD). The background O.D. value is calculated from wells in the absence of anti-human IgE mAb. For those test samples considered to be positive for anti-human IgE Abs a concentration will be assigned with reference to the standard curve.

FcεRIα binding assay (Protein A plates)

8.

9. Introduction

In this assay, a recombinant form of the ectodomain of the alpha chain of the high affinity receptor for IgE (alpha ectodomain) is utilised to bind chimaeric IgE. The carboxyl terminus of the alpha ectodomain is fused to a human IgG1 Fc sequence.

This enables the recombinant molecule to be bound to protein A coated microtitre plates via the Fc region. Hence, the majority of the alpha ectodomain molecules should be available for binding ligand and provides a system for the analysis of IgE - receptor interactions. The format described below is aimed at detecting the (high affinity) receptor blocking activity of anti-IgE antibodies.

Materials

Materials	**Source (Product Code)**
Protein A coated plates	Pierce (15130EE)
Reagent reservoirs	Costar (4870)
Recombinant α-ecto-Ig Fusion protein	In house
Human/mouse chimaeric IgE	Serotec (MCA 333B)
Goat anti-mouse lambda chain HRPO linked antibody	Harlan (SBA 1060-05)
Pig serum	Serotec (C15SC)
Bovine serum albumin (fraction V)	Sigma (A-2153)
Phosphate buffered saline	Sigma (P-4417)
Tween-20	Sigma (P-1379)
Phosphate-citrate buffer with sodium perborate	Sigma (P-4922)
0-phenylene diamine (OPD) tablets	Sigma (P-7288)
25% v/v sulphuric acid	Fisons (H/0564/21)

10.

11. Equipment

Equipment	**Supplier (Code)**
Multichannel pipette	Finnpipette or similar

Ultrawash Plate washer	Dynex Technologies
1296-001Delfia Plateshake	Wallac
MRX Plate reader	Dynatech

12.

13. Method

ELISA protocol for detection of binding of IgE to the alpha chain ectodomain of the high affinity receptor

13.1 Coat protein A plates with 100µl/well α-ecto-Ig fusion protein diluted to 0.25µg/ml in blocking buffer (PBS/5% BSA/0.05% Tween-20). Incubate 1 hour at 37°C.

13.2

13.3 Dilute chimaeric IgE to 0.03125µg/ml in 10% pig serum. Dilute anti-IgE antibody to appropriate test concentration(s) in this IgE solution. Incubate 1 hour at room temperature.

13.4

13.5 Wash plates three times with PBS/0.05% Tween-20 using plate washer.

13.6

13.7 Add 100µl/well of IgE:anti-IgE solution (quadruplicates of each anti-IgE concentration are assayed). Incubate 1 hour at 37°C.

13.8

13.9 Wash plates three times with PBS/0.05% Tween-20 using plate washer.

13.10

13.11 Add 100µl/well of goat anti-mouse lambda chain HRPO conjugated antibody diluted 1:6000 dilution in blocking buffer. Incubate 1 hour at 37°C.

13.12

13.13 Wash plates three times with PBS/0.05% Tween-20 using plate washer.

13.14

13.15 Add 200µl/well of OPD substrate and incubate at room temperature in the dark for 2-10 minutes. Stop the reaction by the addition of 25µl 25% H_2SO_4. Mix stopped reactions on plateshaker – SLOW speed. Read OD at 490nm.

14. Treatment of Results

A figure for the percentage of inhibition of binding of IgE to its receptor can be calculated. A maximum binding value for IgE is determined from the average of a set of wells that contained IgE in 10% pig serum alone (i.e no anti-IgE).

The % inhibition value is calculated thus:
(max IgE value – average of anti-IgE replicates)/max IgE value x 100

FcεRIα binding assay (Clipped ectodomain)

This assay is essentially identical to the previous assay except that the ectodomain/IgG construct is treated with the proteolytic enzyme Factor X to cleave the two moieties. The IgG Fc moiety is removed using protein A beads, and the Factor X is removed using strepatavidin beads, thus leaving an essentially pure alpha chain ectodomain product. In this assay format, the alpha ectodomain is bound directly to plastic microtitre plates, all other assay details are as described above.

CD23-binding assay.

Method

Harvest, wash and re-suspend RPMI 8866 cells at 10^6/ml in sterile PBS. Add 500 µl to a 5ml FACS tube.

Add 500 µl chimeric human IgE (Serotec MCA333B) diluted in PBS at x2 the required final dilution and add to the cells. Incubate on ice for 1hr.

Note: Where blocking antibodies are to be tested the chimeric IgE is incubated with the blocking antibody for 1hr at room temperature prior to addition to the cells.

Wash cells twice by centrifugation at 270g for 5min in an excess of PBS.

Re-suspend cells in 500µl of PBS containing 10 µl of goat anti-human IgE antibody conjugated to FITC (Biosource AHI0508). Incubate on ice in dark for 1 hr.

Repeat wash step 4.3

Re-suspend cells in 500µl of PBS containing 5 µg/ml propidium iodide (Sigma). Briefly vortex to mix.

Collect and analyse 10,000 live gated events by flow cytometry

HBA Assay

15.

16. Materials

Material	**Source (Product Code)**
Human blood	In-house from allergic donor with defined sensitivity to *Lol p* I
EDTA	BDH (100935V)
Ficoll-Paque	Pharmacia (17-0840-02)
HEPES buffered Hanks' balanced salt solution (HBH)	In-house (prepared according to Document No. srgt23
Human serum albumen (HSA)	Sigma (A8763)
Lol p I soluble extract	ALK UK (223204)
Immunotech histamine EIA kit	Serotec (2562)

17.

Equipment

18.

Equipment	**Supplier (Code)**
50 ml plastic disposable syringes	Becton Dickinson
19 or 21 gauge sterile hypodermic needles	Becton Dickinson
Blood collection tubes	Not critical
96-well V-bottom cell culture plates	Costar (3894)
Benchtop centrifuge capable of accepting 50ml tubes, and giving 500Xg	Not critical
MRX ELISA plate reader	Dynex Technologies

19. Method

Blood collection and cell preparation.

19.1 Blood is collected by venepuncture into tubes containing 0.1 volumes 2.7% EDTA, pH 7.0. It is then diluted 1/2 with an equal volume of HBH containing 0.1% HSA (HBH/HSA).

19.2 The resulting cell suspension is carefully layered over 50% volume Ficoll-Paque and centrifuged at 400g for 30 minutes at room temperature. The peripheral blood mononuclear cell (PBMC) layer at the interface is collected and the pellet is discarded.

19.3 The cells are washed once in HBH/HSA, counted, and re-suspended in HBH/HSA at a cell density of 2.0×10^6 per ml.

19.3.1 Cell Incubations

19.4

100µl cell suspension are added to wells of a V-bottom 96-well plate containing 100µl diluted test sample. Each test sample is tested at a range of dilutions with 6 wells for each dilution.

Well contents are mixed briefly using a plate shaker, before incubation at 37°C for 30 minutes with shaking at 120 rpm.

For each serum dilution 3 wells are triggered by addition of 10µl *Lol p* I extract (final dilution 1/10000) and 3 wells have 10µl HBH/HSA added for assessment of anaphylactogenicity.

Well contents are again mixed briefly using a plate shaker, before incubation at 37°C for a further 30 minutes with shaking at 120 rpm.

Incubations are terminated by centrifugation at 500g for 5 min. Supernatants are removed for histamine assay using the standard histamine method provided with the kit.

Control wells containing cells without test sample are routinely included to determine spontaneous and triggered release. Wells containing cells + 0.05% Igepal detergent are also included to determine total cell histamine.

Treatment of Results

Anaphylactogenesis assay

Histamine release due to test samples =

% histamine release from test sample treated cells – % spontaneous histamine release.

Blocking assay

The degree of inhibition of histamine release can be calculated using the formula:

% inhibition

= 1 -(histamine release from test sample treated cells*) x 100
(histamine release from antigen stimulated cells*)

* Values corrected for spontaneous release.

Protocol For the Panning of Phage Libraries Against Biotinylated Antibody

Day 1 (First Round Elution)

1. Take 500µl (5mg) of M280 streptavidin beads. Resuspend the beads in 1ml 4%MPBS (4%Marvel dried milk in PBS) and incubate on a rotating turntable at R.T. for 1hour. After blocking, pellet the beads (13000 rpm for 15secs) and resuspend in 0.5ml of 2%MPBS.

2. Dilute $5x10^{11}$ pfu of phage into 1.5ml of 2%MPBS. Incubate at R.T. for 30mins. Add 10µg of biotinylated antibody in a minimum volume. Place on a rotating turntable for 1hour at R.T.

3. Use 0.7ml of K91 cells to inoculate 11ml of NZY. Grow shaking at 225rpm, 37°C until $OD_{600} = 1.8$.

4. Add 0.5ml of blocked beads from step 1 to the mixture from step 2 and incubate on a rotating turntable for 30mins at R.T. Wash the beads using 3x1ml PBS-0.1%Tween and 3x1ml PBS.

5. Resuspend the pellet in 0.5ml of 0.1M HCl, 1mg/ml BSA, pH adjusted to 2.2 using glycine. Incubate at R.T. for 15mins, pellet the beads, remove the supernatant **AND KEEP**. Neutralise using 1ml of 1M Tris pH8.5.

6. Wash the beads x3 using 1ml PBS and resuspend the pellet in 0.5ml of 0.1M TEA (triethyl acetate). Incubate at R.T. for 15mins, pellet the beads, remove the supernatant **AND KEEP**. Neutralise using 1ml of 1M Tris pH8.5.

7. When the cells have reached OD_{600} ~1.8 shake for 10mins at 50rpm and add half of the pooled, eluted phage. Leave at R.T. for 10mins with occasional swirling.

8. Add 90ml of NZY medium to the culture and 0.2µg/ml tetracycline. Grow for 30mins at 225rpm for 30mins.

9. Take 5µl of the culture and titer as plaques on NZY plates. Increase the tetracycline concentration of the culture to 15µg/ml.

10. Grow the culture overnight at 225rpm, 37°C.

Day 2 (First Round Amplification)

11. Spin the overnight culture at 10000rpm for 10minutes. Remove the supernatant carefully and add 0.15 volumes of 16.7%PEG/3.3M NaCl. Allow phage to precipitate for 2 hours at 4°C.

12. Spin both PEG precipitates at 15000rpm for 15mins. Remove the supernatant thoroughly and resuspend the pellet in 1ml PBS.

13. Centrifuge for 10mins at 13000rpm to pellet residual cells. Transfer the supernatant to a fresh tube and reprecipitate using 0.15 volumes of 16.7%PEG/3.3M NaCl for 1hour on ice.

14. Centrifuge at 13000rpm for 15mins and discard the supernatant. Resuspend the pellet in 200µl of PBS, 0.02% azide.

15. Titer the purified phage as plaques on NZY plates.

Day 3 (Second Round Elution)

1. Take 500µl (5mg) of M280 streptavidin beads. Resuspend the beads in 1ml 4%MPBS and incubate on a rotating turntable at R.T. for 1hour. After blocking, pellet the beads (13000 rpm for 15secs) and resuspend in 0.5ml of 2%MPBS.

2. Dilute $5x10^{11}$ pfu of phage into 1.5ml of 2%MPBS. Incubate at R.T. for 30mins. Add 10µg of biotinylated antibody in a minimum volume. Place on a rotating turntable for 1hour at R.T.

3. Use 0.7ml of K91 cells to inoculate 11ml of NZY. Grow shaking at 225rpm, 37°C until $OD_{600} = 1.8$.

4. Add 0.5ml of blocked beads from step 1 to the mixture from step 2 and incubate on a rotating turntable for 30mins at R.T. Wash the beads using 3x1ml PBS-0.1%Tween and 3x1ml PBS.

5. Resuspend the pellet in 0.5ml of 0.1M HCl, 1mg/ml BSA, pH adjusted to 2.2 using glycine. Incubate at R.T. for 15mins, pellet the beads, remove the supernatant **AND KEEP**. Neutralise using 1ml of 1M Tris pH8.5.

6. Wash the beads x3 using 1ml PBS and resuspend the pellet in 0.5ml of 0.1M TEA. Incubate at R.T. for 15mins, pellet the beads, remove the supernatant **AND KEEP**. Neutralise using 1ml of 1M Tris pH8.5.

7. When the cells have reached OD_{600} ~1.8 shake for 10mins at 50rpm. Pour 10ml into a flask and add half of the pooled, eluted phage. Leave at R.T. for 10mins with occasional swirling.

8. Add 90ml of NZY medium to the culture and 0.2µg/ml tetracycline. Grow for 30mins at 225rpm for 30mins.

9. Take 5µl of the culture and titer as plaques on NZY plates. Increase the tetracycline concentration of the culture to 15µg/ml.

10. Grow the culture overnight at 225rpm, 37°C.

Day 4 (Second Round Amplification)

11. Spin the overnight culture at 10000rpm for 10minutes. Remove the supernatant carefully and add 0.15 volumes of 16.7%PEG/3.3M NaCl. Allow phage to precipitate for 2 hours at 4°C.

12. Spin both PEG precipitates at 15000rpm for 15mins. Remove the supernatant thoroughly and resuspend the pellet in 1ml PBS.

13. Centrifuge for 10mins at 13000rpm to pellet residual cells. Transfer the supernatant to a fresh tube and reprecipitate using 0.15 volumes of 16.7%PEG/3.3M NaCl for 1hour on ice.

14. Centrifuge at 13000rpm for 15mins and discard the supernatant. Resuspend the pellet in 200µl of PBS, 0.02% azide.

15. Titer the purified phage as plaques on NZY plates.

Day 5 (Third Round Elution)

1. Take 100µl (2mg) of M280 streptavidin beads. Resuspend the beads in 1ml 4%MPBS and incubate on a rotating turntable at R.T. for 1hour. After blocking, pellet the beads (13000 rpm for 15secs) and resuspend in 0.2ml of 2%MPBS.

2. Dilute $5x10^{11}$ pfu of phage into 1.5ml of 2%MPBS. Incubate at R.T. for 30mins. Add 5µg of biotinylated antibody in a minimum volume. Place on a rotating turntable for 1hour at R.T.

3. Use 0.7ml of K91 cells to inoculate 11ml of NZY. Grow shaking at 225rpm, 37°C until $OD_{600} = 1.8$.

4. Add 0.2ml of blocked beads from step 1 to the mixture from step 2 and incubate on a rotating turntable for 30mins at R.T. Wash the beads using 3x1ml PBS-0.1%Tween and 3x1ml PBS.

5. Resuspend the pellet in 0.5ml of 0.1M HCl, 1mg/ml BSA, pH adjusted to 2.2 using glycine. Incubate at R.T. for 15mins, pellet the beads, remove the supernatant **AND KEEP**. Neutralise using 1ml of 1M Tris pH8.5.

6. Wash the beads x3 using 1ml PBS and resuspend the pellet in 0.5ml of 0.1M TEA. Incubate at R.T. for 15mins, pellet the beads, remove the supernatant **AND KEEP**. Neutralise using 1ml of 1M Tris pH8.5.

7. When the cells have reached OD_{600} ~1.8 shake for 10mins at 50rpm. Pour 10ml into a flask and add half of the pooled, eluted phage. Leave at R.T. for 10mins with occasional swirling.

8. Add 90ml of NZY medium to the culture and 0.2µg/ml tetracycline. Grow for 30mins at 225rpm for 30mins.

9. Take 5µl of the culture and titer as plaques on NZY plates. Increase the tetracycline concentration of the culture to 15µg/ml.

10. Grow the culture overnight at 225rpm, 37°C.

Day 7 (Fourth Round Elution)

1. Take 100µl (1mg) of M280 streptavidin beads. Resuspend the beads in 1ml 4%MPBS and incubate on a rotating turntable at R.T. for 1hour. After blocking, pellet the beads (13000 rpm for 15secs) and resuspend in 0.1ml of 2%MPBS.

2. Take 75µl of phage from both the third round acid and base elutions and block with 1.5ml of 2%MPBS. Incubate at R.T. for 30mins. Add 1µg of biotinylated antibody in a minimum volume. Place on a rotating turntable for 1hour at R.T.

3. Add 0.1ml of blocked beads from step 1 to the mixture from step 2 and incubate on a rotating turntable for 30mins at R.T. Wash the beads using 3x1ml PBS-0.1%Tween and 3x1ml PBS.

4. Resuspend the pellet in 0.5ml of 0.1M HCl, 1mg/ml BSA, pH adjusted to 2.2 using glycine. Incubate at R.T. for 15mins, pellet the beads, remove the supernatant **AND KEEP**. Neutralise using 1ml of 1M Tris pH8.5.

5. Wash the beads x3 using PBS and resuspend the pellet in 0.5ml of 0.1M TEA. Incubate at R.T. for 15mins, pellet the beads, remove the supernatant **AND KEEP**. Neutralise using 1ml of 1M Tris pH8.5.

6. Take 10µl of the eluted phage and plate as plaques on NZY plates. Pick the plaques into NZY-TET for analysis.

Example 2, Characterisation of novel anti-human IgE monoclonal antibodies

Effects on the interaction of IgE with FcεRI

PTmAb0011 is a mouse monoclonal antibody with specificity for human IgE, showing no cross-reactivity with other human Ig isotypes or rat/mouse IgE. PTmAb0011 binds to both native and heat-treated IgE, when bound to an ELISA plate in a random orientation, indicating that its recognition site on IgE is not heat labile. PTmAb0011 also recognises IgE when bound via antigen to the ELISA plate. Importantly this mAb can completely block the interaction between human IgE and

the ε-chain binding component of the high affinity IgE receptor (*FcεRI*). However, this mAb still recognises human IgE when pre-bound to FcεRI, indicating that the mAb binding site is not lost upon receptor binding.

Methods

19.5 Analysis of IgE binding properties of PTmAb0011 by ELISA

ELISA plates were coated with human chimaeric IgE, myeloma IgE, human Ig isotypes or rodent IgE (1μg/ml in pH 9.6 carbonate/bicarbonate coating buffer). For antigen orientated ELISAs, NP-BSA was coated at a saturating concentration prior to the addition of chimaeric IgE (1μg/ml). Alternatively, soluble human FcεRI α-chain was coated (0.25μg/ml) followed by chimaeric IgE. After blocking non-specific binding sites with PBS/0.05% Tween-20 containing 5% w/v BSA, PTmAb0011 or an isotype control mAb was added, with dilutions being made in PBS/0.05% Tween-20, 1% w/v BSA. After washing the plate, bound PTmAb0011 was detected with sheep anti-mouse IgG-HRP (1/4000) and bound HRP conjugated antibody detected with OPD substrate at 490nm.

α-chain blocking assay

ELISA plates were coated with FcεRI α-chain in carbonate/bicarbonate pH 9.6 coating buffer and then blocked with PBS/0.05% Tween-20/ 5% BSA. During this time chimaeric IgE was pre-incubated with PTmAb0011 or isotype matched control antibody, prior to addition to the plates. After adding the mixture to the plates, bound IgE was detected after a 1-hour incubation with HRP-anti-mouse λ-light chain antibody. Bound HRP antibody was revealed with OPD substrate.

Results

Figures 6A and 6B illustrate that PTmAb0011 binds to both human/mouse chimaeric IgE and human myeloma IgE when bound to an ELISA plate in a random orientated manner. Binding is dose dependent with maximal binding activity seen at 0.63μg/ml of PTmAb0011. In contrast, no such binding was seen with the isotype matched control mAb. Similarly, binding to antigen orientated IgE (i.e IgE bound to plate

bound NP-BSA) is dose dependent with maximum activity evident at 0.63μg/ml (Fig 6C).

PTmAb0011 was also analysed for its ability to recognise chimaeric IgE following heat treatment at 56°C for a range of time periods. Figure 6D shows that the binding capacity of PTmAb0011 for IgE is unaffected by heat treatment.

The mAb characterisation was further extended to determine whether PTmAb0011 was able to inhibit the interaction of IgE with the α-chain component of the high affinity IgE receptor (Fig 7). Pre-incubation of IgE with PTmAb0011 prior to addition to plate bound FcεRI α-chain, resulted in a dose dependent inhibition of the interaction of IgE with FcεRI α-chain. Where PTmAb0011 was used at 10μg/ml 95% inhibition was seen, with no significant inhibition seen at 0.05μg/ml levels. The isotype matched control mAb showed no significant inhibitory properties over an equivalent dose range.

PTmAb0011 was also assessed for its ability to recognise IgE when pre-bound to FcεRI α-chain. Figure 8 shows that PTmAb0011 recognises FcεRI α-chain associated IgE in a dose dependent manner, with maximal binding achieved at 0.31μg/ml. No significant binding was seen with the isotype matched control mAb.

Summary

PTmAb0011 is a mouse monoclonal antibody which shows specific binding for human IgE *vs.* other human Ig isotypes and does not recognise rodent IgE.

PTmAb0011 shows equivalent recognition of native human/mouse chimaeric IgE and myeloma IgE. Also, recognition of chimaeric IgE is comparable for both plate bound (random orientated) and antigen orientated IgE.

The binding activity of PTmAb0011 for IgE is not affected by heat treatment of IgE at 56°C, indicating that its epitope is not heat labile.

PTmAb0011 is a potent inhibitor of the interaction of IgE with soluble Fc?RI ?-chain when immobilised on an ELISA plate.

PTmAb0011 differs from rhuMabE-25 in that it is able to recognise IgE once bound to FcεRI α-chain.

II *Effects on the interaction of IgE with FcεRII*

We have identified anti-IgE mAbs that either promote (PTmAb0011) or inhibit (PTmAb0017) the binding of IgE to the low affinity IgE receptor (FcεRII/CD23) on RPMI 8866 cells. Using pre-formed complexes of mAb and IgE, we demonstrated the potentiating or inhibitory effects of such anti-IgE mAbs on the binding of IgE to CD23 using flow cytometry. We determined that PTmAb0011 recognised soluble IgE more efficiently than IgE pre-bound to CD23. Furthermore, PTmAb0011 also promoted the binding of chimaeric IgE to primary human B-cells. Based upon these observations, we examined the effects of anti-IgE mAbs on the secretion of IgE from human B-cells co-stimulated with IL-4 and anti-CD40 by ELISA. We found that incubation of primary human B-cells with PTmAb0011 significantly reduced the levels of secreted IgE.

Methods

Isolation of peripheral blood mononuclear cells

Heparinised human blood was diluted 1:1 in RPMI 1640 medium prior to layering on to ficoll-hypaque and centrifugation at 400xg for 30min. Mononuclear cells were harvested from the interphase and cultured in RPMI 1640 medium supplemented with 10% Human AB serum. Enhanced CD23 expression was promoted on constituent B-cells by supplementing the culture medium with 10 ng/ml hIL-4 and 1 μg/ml anti-CD40 antibody (clone 5C3) for a 24 hour period prior to the initiation of cell binding studies.

19.6 CD23 binding assay

This assay was performed on either RPMI 8866 cells or primary human B-cells; two formats were used, one for the detection of mAbs that bind to IgE associated with Fc?RII, and a second that analysed whether the mAbs interfered with IgE association with Fc?RII. For the first assay cells were loaded with chimaeric IgE (1 μg/ml) for an hour on ice in PBS, 1% FBS, 0.1% NaN_3. Excess IgE was removed and anti-IgE mAb added. Bound mAb was elucidated with FITC-conjugated rat anti-mouse IgG_1

antibody. For the second assay, chimaeric IgE (1 μg/ml) was pre-incubated with anti-IgE mAb for an hour at room temperature, with gentle mixing, prior to addition to the cells. The mixture was incubated with the cells for an hour on ice and then washed to remove unbound IgE. Bound IgE was detected with FITC-goat anti-human IgE or bound anti-IgE mAb was detected with FITC-conjugated rat anti-mouse IgG_1 antibody. Where studies were performed on PBMCs, constituent B-cells were identified with a PE-conjugated anti-CD19 antibody. Samples were analysed by flow cytometry.

Analysis of IgE secretion from primary human B-cells

PBMCs were plated at $2x10^5$ cells per well in 96 U-well plates, in medium supplemented with both IL-4 and anti-CD40. PTmAb0011 or isotype matched control mAb was added and cells incubated for 14 days prior to harvesting of supernatants for IgE analysis.

19.7 Analysis of secreted IgE by ELISA

Total IgE levels were measured by coating ELISA plates with rabbit anti-human IgE antibody (10μg/ml) in 0.5M carbonate/bicarbonate buffer (pH9.6). Washed plates were blocked with PBS, 0.05% Tween 20, 5% BSA. Both cell supernatants and IgE standard were incubated with saturating amounts of PTmAb0011 (10μg/ml) for an hour at room temperature prior to addition to the ELISA plate to allow for IgE/anti-IgE complexes to be formed. Following incubation and washing steps, bound IgE was detected with HRP-sheep anti-human IgE, followed by OPD substrate. Levels of IgE in the cell supernatants were then estimated relative to the standard curve.

Results

Analysis of the effects of anti-IgE antibodies on IgE binding to the low affinity IgE receptor FcεRII on RPMI 8866 cells.

IgE was pre-incubated with either PTmAb0011 or PTmAb0017 over a dose range from 10 μg/ml to 0.5 μg/ml and examined for their effects on subsequent IgE binding to FcεRII on the human B-cell line 8866. Figure 9A illustrates that pre-incubation of

IgE with PTmAb0011 enhances IgE binding to FcεRII. This enhancement in binding shows a bell-shaped distribution. In contrast, PTmAb0017 showed a potent and dose dependent inhibition in IgE binding to FcεRII (Fig 9B).

Analysis of the ability of PTmAb0011 to recognise FcεRII associated IgE

We extended the studies described above to examine whether PTmAb0011 could recognise FcεRII bound IgE. Figure 10A illustrates that PTmAb0011 shows very weak recognition of receptor bound IgE. As a control for this experiment, PTmAb0011 was pre-incubated with IgE, prior to addition to the FcεRII positive cells (Fig 10B). Under these experimental conditions it is clear that PTmAb0011 can be detected on the surface of the RPMI 8866 cells, but only if it exists in complexes with IgE prior to addition to the cells.

Analysis of the effects of anti-IgE antibodies on IgE binding to the low affinity IgE receptor FcεRII on primary B-cells

In figure 9A we have shown that PTmAb0011 enhances IgE binding to FcεRII expressed on the human B-cell line RPMI 8866 cells. We extended these studies to examine whether the same increase in binding was detected on primary B-cells. From figure 11 it is clear that PTmAb0011 does enhance IgE binding to FcεRII on primary B-cells in an equivalent manner to that seen on the RPMI 8866 cell line.

Effects of PTmAb0011 on IgE secretion from primary human B-cells

The studies described above have illustrated that the anti-IgE PTmAb0011 can enhance IgE binding to FcεRII on a human B-cell line and on primary human B-cells. The specificity of this interaction was confirmed by competition studies with an anti-FcεRII mAb (data not shown). We therefore sought to extend the results gained from these cell based binding studies to examine the effects of PTmAb0011 on IgE secretion from primary human B-cells. Peripheral blood mononuclear cells were cultured with PTmAb0011, in the presence of additional IL-4 and anti-CD40 antibody to promote B-cell isotype switch to IgE secretion. An ELISA assay was developed that allowed for measurement of total IgE levels, that is free IgE and PTmAb0011 complexed IgE. To achieve such quantitation secreted IgE was pre-incubated with

saturating levels of PTmAb0011 to allow for all of the IgE to be complexed. The total IgE within the tissue culture supernatant was quantitated relative to a standard curve of IgE that had also been complexed with saturating levels of PTmAb0011. Figure 12 illustrates that in three different donors, incubation of primary B-cells with PTmAb0011 (1 μg/ml) resulted in a significant reduction in the total levels of secreted IgE. No such inhibition was seen with the isotype matched control antibody.

Summary

PTmAb0011 causes a dose dependent increase in IgE association with FcεRII on the surface of a human B-cell line and also on primary human B-cells. This enhanced binding could be due to increased occupancy of FcεRII on the surface of the B-cells, or due to the docking of IgE-PTmAb0011 complexes on to FcεRII, or a combination of the both mechanisms.

PTmAb0011 shows very weak recognition of IgE once bound to FcεRII, suggesting that its epitope is either masked or that its conformation is changed upon receptor binding.

Incubation of primary B-cells with PTmAb0011, under conditions that promote IgE secretion, resulted in a reduction in the levels of secreted IgE. It is postulated that this reduction may be due to an increase in negative signalling via FcεRII due to enhanced occupancy of the receptor and/or an increase in the affinity of the interaction due to docking of IgE/PTmAb0011 complexes.

Functional effects in human basophils, human lung mast cells and RBL cells.

It is generally accepted that antibodies able to cross-link FcεR1-bound IgE molecules on the surface of mast cells and basophils will trigger degranulation of the cell. We have explored the anaphylactogenic properties of a number of anti-human IgE monoclonal antibodies in human basophils, human lung mast cells and RBL cells transfected with human FcεR1 α-chain. While most antibodies were able to trigger histamine release from these cells, we identified a monoclonal antibody, PTmAb0011, that was able to bind to receptor-bound IgE without causing significant histamine release. Furthermore, PTmAb0011 blocked the passive sensitisation of non-allergic basophils by preventing the binding of IgE to FcεR1α, and also inhibited Lolp1-

triggered histamine release in Lolp1 sensitive basophils. The functional profile of PTmAb0011 demonstrates that this or similar antibodies are useful in the treatment of allergic disease.

19.8 Determination of histamine release from human basophils

Two assay formats were adopted. PBMC from non-allergic donors were passively sensitised with 1µg/ml chimeric IgE for 30min at 37°C, washed and treated with monoclonal antibodies for 30min at 37°C. Alternatively PBMC from LolP1-sensitive donors were treated directly with monoclonal antibodies for 30min at 37°C. Reactions were terminated by centrifugation. Histamine release in cell supernatants was determined by specific immunoassay (Immunotech 2562). Total cellular histamine content was determined in cells lysed with 0.5% Igepal detergent.

19.9 Basophil blocking assay

The ability of anti-IgE antibodies to block binding of chimeric IgE to FcεR1 on human basophils was determined by incubation of PBMC from non-allergic donors with chimeric IgE in the presence of monoclonal antibodies and IL-3 for 30min at 37°C. Cells were washed and histamine release was triggered with NP-BSA for a further 30min at 37°C. Reactions were terminated by centrifugation and histamine release measured as above.

19.10 Allergic basophil inhibition assay

The ability of anti-IgE antibodies to inhibit allergen-triggered degranulation was investigated by pre-incubating PBMC from LolP1-sensitive donors with monoclonal antibodies for 30min at 37°C prior to triggering with LolP1

***19.11* Determination of tryptase release from human lung mast cells**

Crude mast cell suspensions were prepared from human lung tissue by enzymatic digestion with a cocktail comprising hyaluronidase, pronase and DNAse. Cells were either used directly or pre-sensitised with chimeric IgE prior to treatment with anti-IgE antibodies. Mast cell degranulation was determined by colorimetric assay of the granule enzyme tryptase.

Determination of β-hexosaminidase release from RBL cells transfected with human FcεR1α

Transfected cell line RBL J41 was obtained from Dr B. Helm, University of Sheffield. Cells were passively sensitised with either mouse monoclonal IgE anti-DNP or human chimeric IgE anti-NP and triggered with anti-human IgE antibodies. Degranulation was measured by the colorimetric assay of β-hexosaminidase release.

Results

Anaphylactogenicity of anti-IgE monoclonal antibodies in human basophils

A number of anti-IgE monoclonal antibodies were assayed for their ability to trigger histamine release from both allergic (figure 13A) and non-allergic (figure 13B) basophils. Most antibodies were able to cause significant histamine release in all donors. However, PTmAb0011 was consistently unable to generate significant histamine release.

Anaphylactogenicity of anti-IgE monoclonal antibodies in human lung mast cells

PTmAb0011 was also unable to release significant amounts of tryptase in both sensitised and non-sensitised human lung mast cells (figures 14A and 14B). Polyclonal anti-human IgE gave 60-70% release in these cells.

Anaphylactogenicity of anti-IgE monoclonal antibodies in RBL cells transfected with human FcεR1α

RBL J41 cells, passively sensitised with chimeric human IgE anti-NP, could be triggered with antigen NP-BSA and with polyclonal anti-human IgE but not with PTmAb0011 (figure 15A). In contrast, when cells were sensitised with mouse IgE anti-DNP, both anti-human IgE antibodies were without effect. The cells could still be triggered by antigen DNP-BSA (figure 15B)

19.11.1.1.1.1 Basophil blocking assay

PTmAb0011 was able to block the binding of IgE to FcεR1 in non-allergic basophils and thus to inhibit subsequent triggering with NP-BSA antigen. The IC_{50} value of this activity was around 60ng/ml (figure 16A)

19.12 Allergic basophil inhibition assay

PTmAb0011 was also able to potently inhibit LolP1-triggered histamine release from allergic basophils with an IC_{50} value of 40ng/ml (figure 16B).

Summary

We have identified a novel anti-human IgE monoclonal antibody, PTmAb0011, that is able to recognise IgE when bound to its high affinity receptor on human basophils without causing significant degranulation.

The lack of anaphylactogenicity of PTmAb0011 has been confirmed in human lung mast cells (figure 2) and in RBL cells transfected with human FcεR1α.

PTmAb0011 is able to block the passive sensitisation of non-allergic basophils by preventing the binding of IgE to FcεR1α.

PTmAb0011 also inhibits LolP1-triggered histamine release in allergic basophils.

The functional profile of PTmAb0011 suggests that this or similar antibodies might be useful in the treatment of allergic diseases.

20.

Claims

1. A pharmaceutical composition for therapeutic/prophylactic treatment of IgE-mediated immune response such as allergy, comprising (A) the anti-IgE antibody termed PtmAb0011, which recognises a region of the human IgE Fc domain and which has the following properties:

i. It is capable of binding human IgE in its non-receptor-bound state (i.e. in free solution or when bound to a solid phase support e.g. an ELISA plate).

ii. It is capable of binding IgE bound to its high affinity receptor (FcεRI).

iii. It will prevent binding of IgE to the high affinity IgE receptor (FcεRI).

iv. It will not prevent IgE binding to the low affinity IgE receptor (FcεRII).

v. It inhibits degranulation of human blood basophils following exposure to antigen.

together with (B) a pharmaceutically and physiologically acceptable carrier, diluent, excipient, adjuvant or the like.

2. A composition according to claim 1 which consists of the monoclonal antibody PTmAb0011 and an adjuvant.

3. An anti-allergy vaccine containing or consisting of a composition according to claim 1 or 2.

4. Use of antibody PTmAb0011 in the manufacture of a medicament for treatment/prophylaxis of an IgE-mediated immune response such as allergy.

5. A method of treatment/prophylaxis of immune response such as allergy, which comprises administering to a patient an anti-allergy-effective amount of a composition according to claim 1 or 2.

6. A method of treatment/prophylaxis of immune response such as allergy, which comprises administering to a patient an anti-allergy-effective amount of a vaccine according to claim 3.

7. Use of anti-IgE antibody PTmAb0011 in an assay to define therapeutically useful sequences from IgE for use as vaccines when coupled to a suitable carrier, such useful sequences being capable of eliciting an anti-IgE immune response in a vaccinated patient.

8. A method of assaying for a therapeutically useful sequence from IgE for use as a vaccine when coupled to a suitable carrier; which method includes the step of contracting a library of possible useful sequences of peptides with anti-IgE antibody PTmAb0011 under antibody binding conditions; identifying one or more sequences which bind to the antibody, and testing said one or more sequences for immunogenicity in vivo.

9. A method according to claim 8 which further includes the step of synthesising an isolated pure polypeptide containing an immunogenic sequence identified by the testing step of the method.

10. A method according to claim 8 or 9 which includes the step of synthesising an isolated and purified anti-IgE immunogenic compound which consists of or includes partly or wholly non-peptidic mimetic of said one or more sequences identified by the testing step of the method.

11. A polypeptide or peptidomimetic produced by the method of claim 9 or 10.

12. A novel anti-IgE antibody which recognises the same epitope or epitopes in the human IgE Fc domain as PTmAb0011, and has the same properties (i)-(v) defined by claim 1 as PTmAb0011.

13. A polypeptide selected from the group consisting of

HCQQVFFPQDYLWCQRG – Seq ID No 1
SCREVWLGGSEMIMDCE – Seq ID No 2
ECNQNLSGSLRHVDLNC – Seq ID No 3
DCEEPMCSPVLLQKLKP – Seq ID No 4
SCREVWLGGSEMIMDCE – Seq ID No 5
RCDQQLPRDSYTFCMMS – Seq ID No 6
SCPAFPREGDLCAPPTV – Seq ID No 7
FCPEPICSPPLSRMTLS – Seq ID No 8
VCDECVSRELAL – Seq ID No 9
WCLEPECAPGLL – Seq ID No 10
VCDECVSRELAL – Seq ID No 11
DCLSKGQMADLC – Seq ID No 12
SCQGREVRRECW – Seq ID No 13
WCREVWLGESETIMDCE – Seq ID 14
ACREVWLGESETIMDCD – Seq ID 15
GCAEPKCWQALHQKLKP – Seq ID 16

14. A compound which is a wholly or partly non peptide mimetic; or N- or C-terminal derivative; or analogue by virtue of conservative amino acid deletion, addition or substitution; or peptidomimetic of a polypeptide according to claim 11 or 13.

15. A composition which comprises a polypeptide according to claim 11 or 13 or a compound according to claim 14 together with an immunogenic carrier and an adjuvant.

16. An anti-allergy vaccine containing or consisting of a composition according to claim 15.

17. Use of a polypeptide according to claim 11 or 13 or a compound according to the claim 14 in the manufacture of a medicament for treatment/prophylaxis of an IgE-mediated immune response such as allergy.

18. A method of treatment/prophylaxis of immune response such as allergy, which comprises administering to a patient an anti-allergy-effective amount of a composition according to claim 15.

19. A method of treatment/prophylaxis of immune response such as allergy, which comprises administering to a patient an anti-allergy-effective amount of a vaccine according to claim 16.

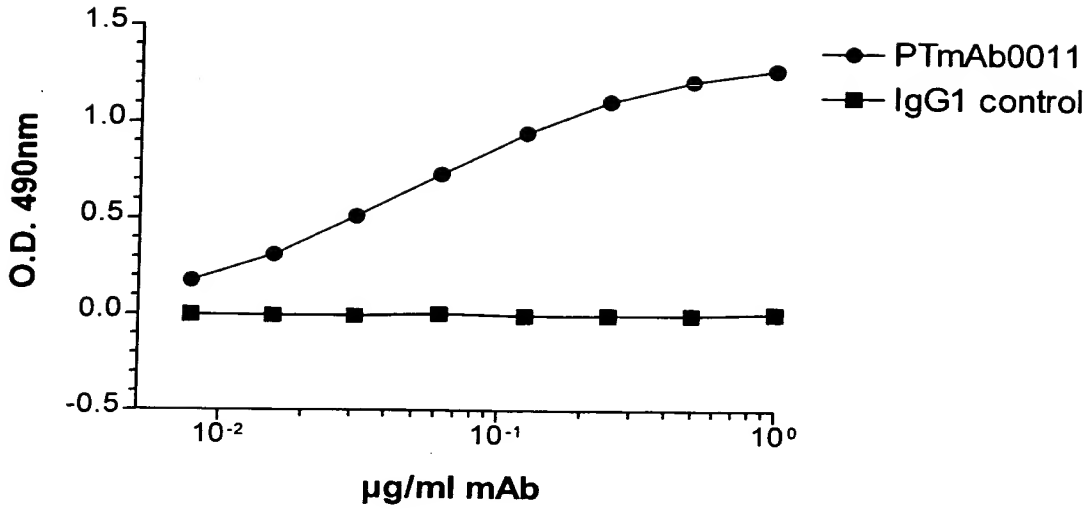

1.5
1.0
0.5
0.0
-0.5
O.D. 490nm
10-2
10-1
100
µg/ml mAb
PTmAb0011
IgG1 control

Fig.1

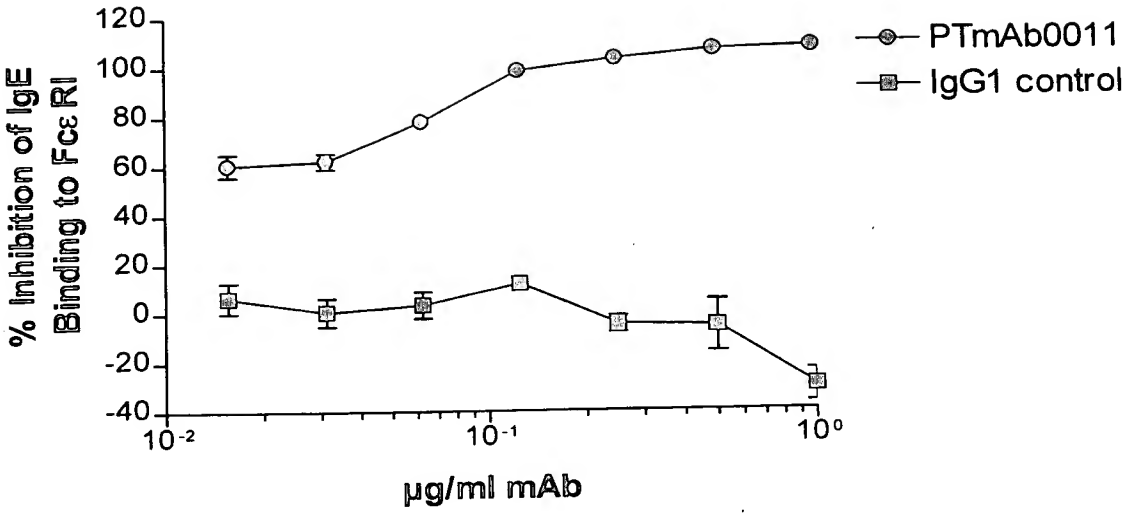

% Inhibition of IgE Binding to FcεRI
120
100
80
60
40
20
0
-20
-40
10-2
10-1
100
µg/ml mAb
PTmAb0011
IgG1 control

Fig.2

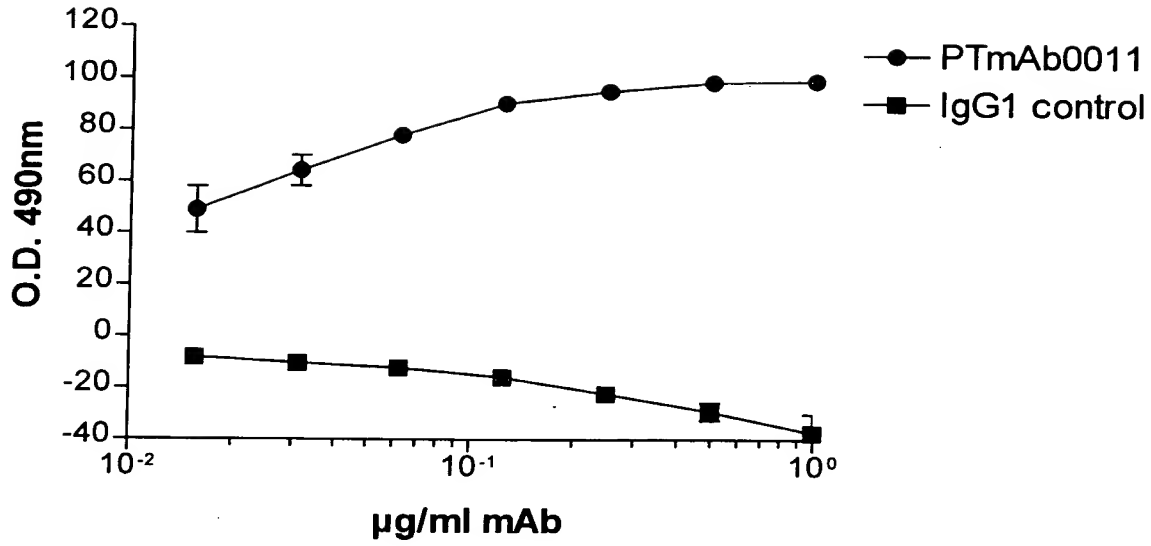

120
100
80
60
40
20
0
-20
-40
O.D. 490nm
10-2
10-1
100
µg/ml mAb
PTmAb0011
IgG1 control

Fig.3

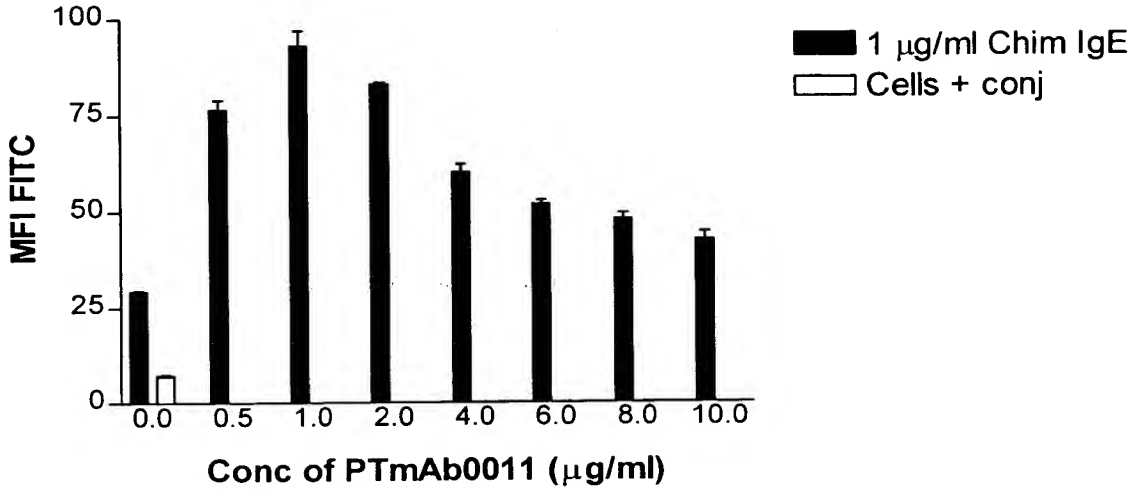

100
75
50
25
0
MFI FITC
0.0
0.5
1.0
2.0
4.0
6.0
8.0
10.0
Conc of PTmAb0011 (μg/ml)
1 μg/ml Chim IgE
Cells + conj

Fig.4

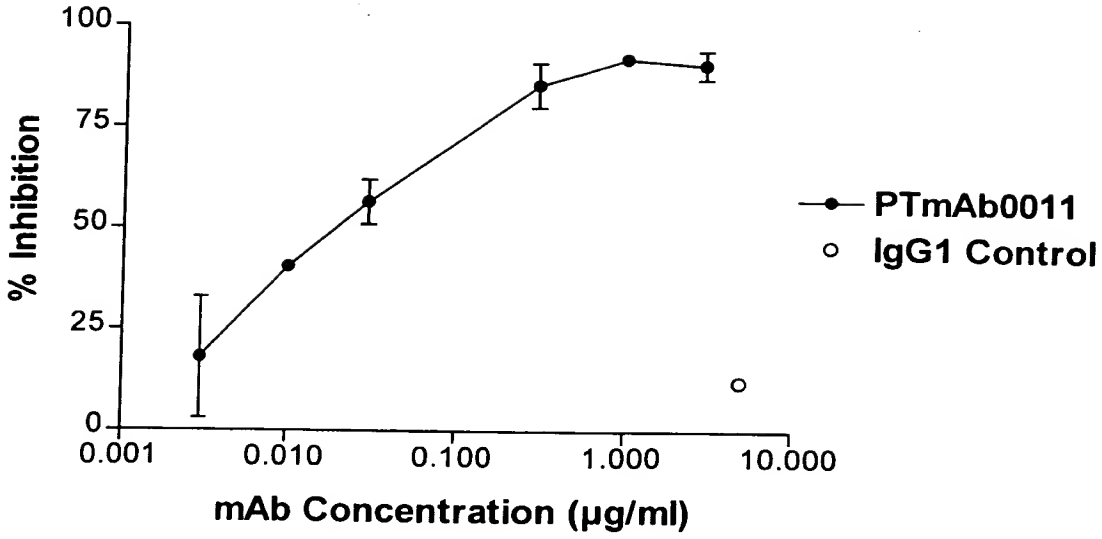
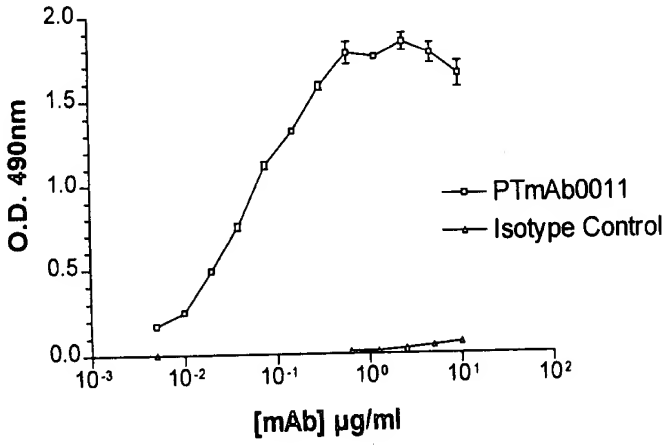
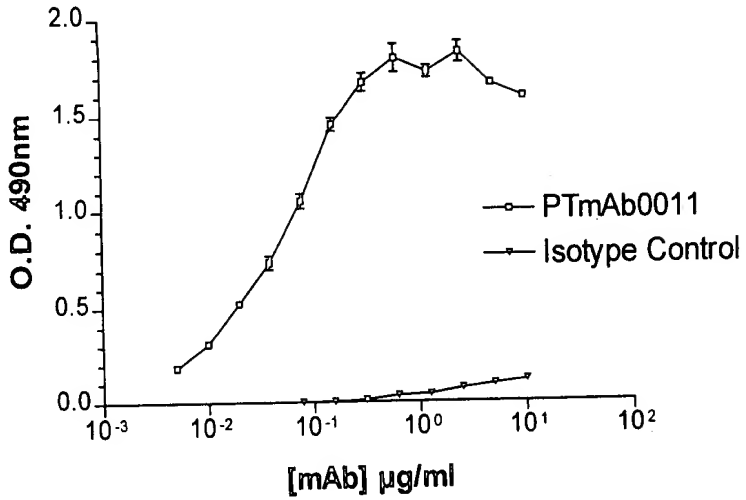

100
75
50
25
0
% Inhibition
0.001
0.010
0.100
1.000
10.000
mAb Concentration (µg/ml)
PTmAb0011
IgG1 Control

Fig.5

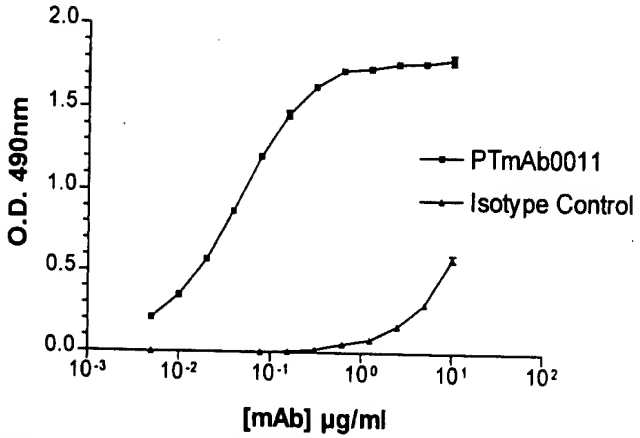
20.1.1.1.1

20.1.1.1.2

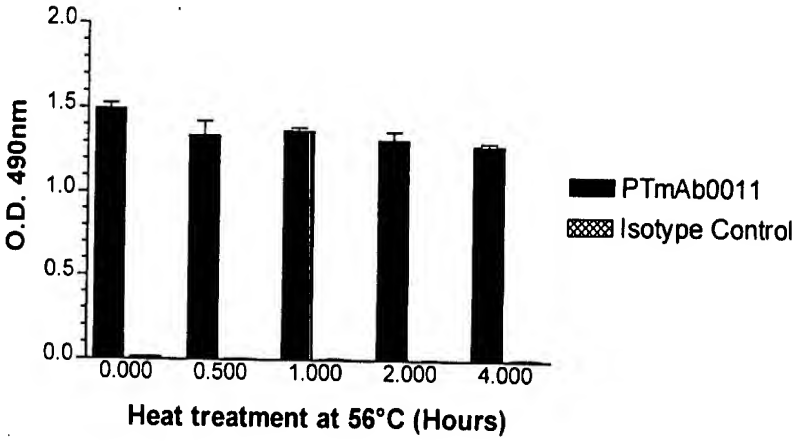




20.1.1.1.3 Figure 6



A) **Binding to Chimaeric IgE**



B) **Binding to Myeloma IgE**

C) **Binding to Antigen Orientated IgE**

D) **Binding to Heat Denatured IgE**

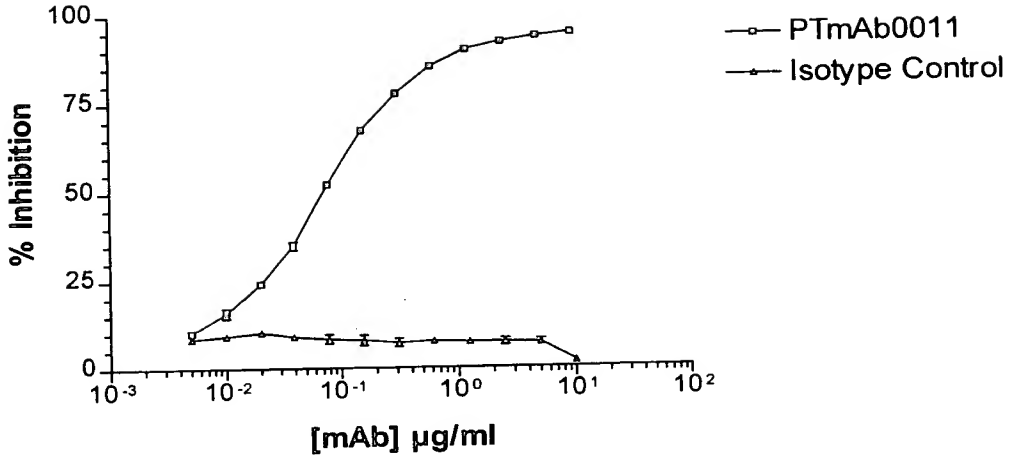


Figure 7 **Inhibition of Binding to FcεR1α**

The assay was carried out as described in the Methods section. Triplicate samples were assayed and the above graph is a typical example.

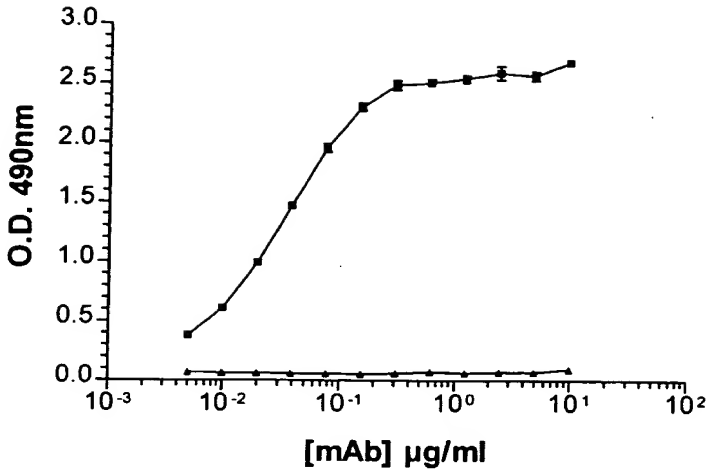


Figure 8 Binding to Receptor Bound IgE

ELISA was performed as described in the Methods section. Triplicate samples were assayed and a representative assay is shown above.

20.1.1.1.4 Figure 9A

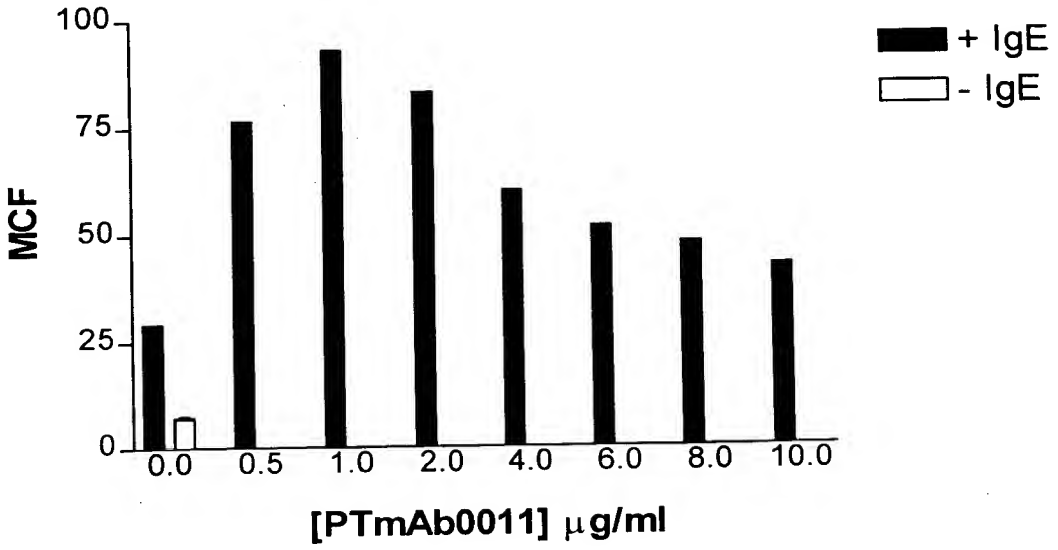


Figure 9B.

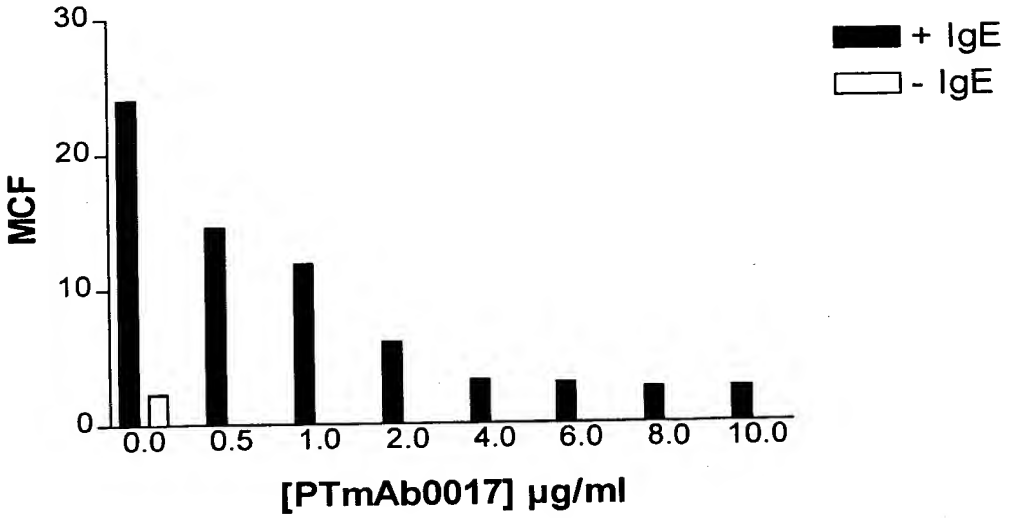
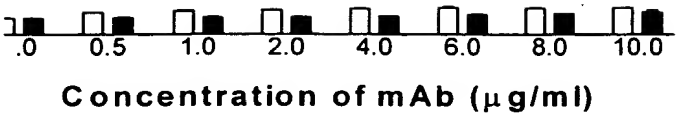


Figure 9- Analysis of the effects of anti-IgE antibodies on IgE binding to FcεRII on RPMI 8866 cells. RPMI 8866 cells ($1x10^6$/ml) were incubated for an hour on ice with chimaeric IgE (1 µg/ml) and anti-IgE mAb (10 to 0 µg/ml). The IgE and anti-IgE were pre-incubated for an hour at room temperature prior to addition to the cells. Bound IgE was detected with FITC-goat anti-human IgE. The results show the mean channel fluorescence (MCF) of duplicate samples as determined by flow cytometric analysis of 10,000 live gated events.



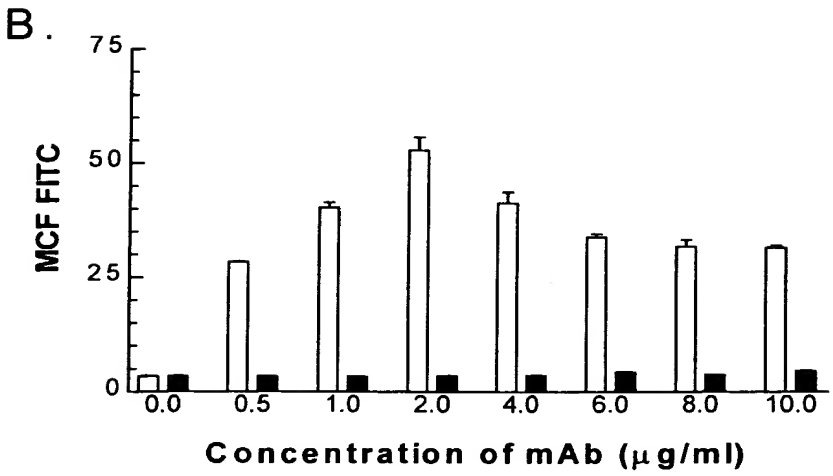


Figure 10 - Analysis of the ability of PTmAb0011 to recognise FcεRII associated IgE. RPMI 8866 cells ($1x10^6$/ml) were incubated with chimaeric IgE (1 μg/ml) for an hour on ice. Excess unbound IgE was removed prior to the addition of PTmAb0011 (10 to 0 μg/ml) (Fig 2A; open) or an equivalent concentration of isotype matched control mAb (solid). Alternatively the IgE and PTmAb0011 (open) or isotype matched control mAb (solid) were pre-incubated together for an hour at room temperature prior to addition to the cells (Fig 2B). Bound PTmAb0011 was detected with FITC-conjugated rat anti-mouse IgG_1 antibody. The results shown are mean channel fluorescence + SEM of triplicate values, determined by flow cytometric analysis of 10,000 live gated events.

B45207
Figure 11A

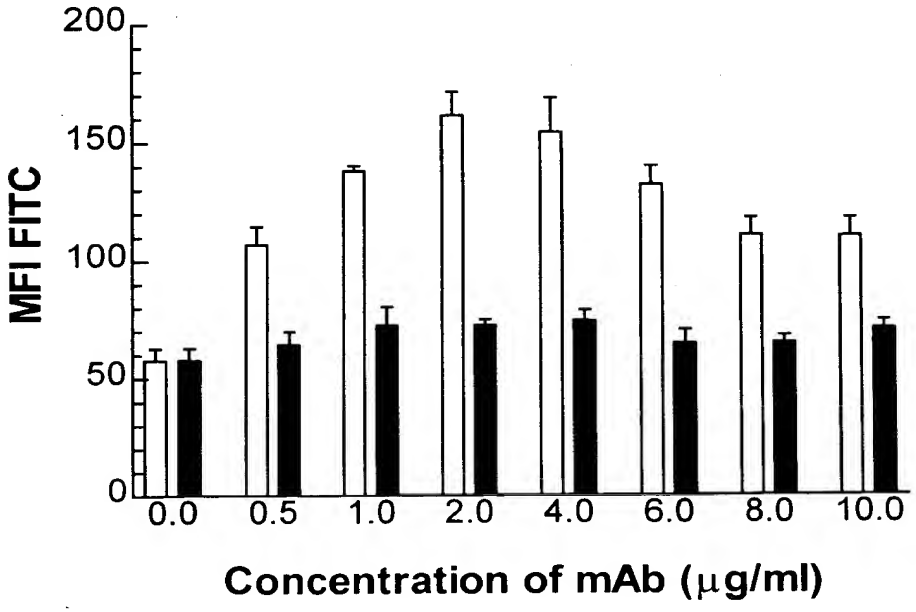
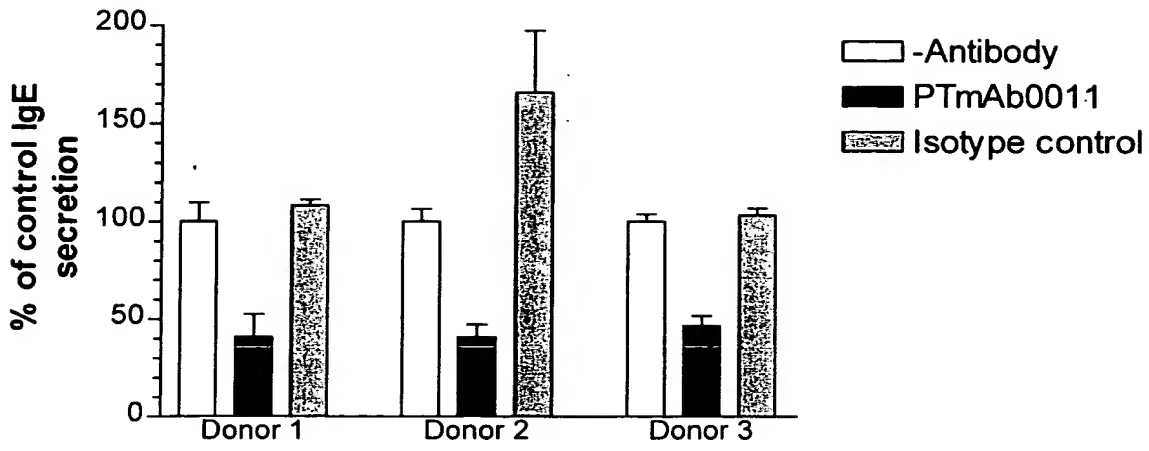


Figure 11 Analysis of the effects of PTmAb0011 on IgE binding to FcεRII on primary human B-cells.

Peripheral blood mononuclear cells ($1x10^6$/ml) were incubated for an hour on ice with chimaeric IgE (1 μg/ml) and anti-IgE mAb (10 to 0 μg/ml; open) or equivalent concentrations of isotype matched control mAb (solid). The IgE and anti-IgE were pre-incubated for an hour at room temperature prior to addition to the cells. Bound IgE was detected with FITC-goat anti-human IgE and the primary B-cells were elucidated with PE-conjugated anti-CD19. The results show the mean channel fluorescence (MCF) of duplicate samples as determined by flow cytometric analysis of 5,000 live gated events.

B45207
Figure 12



20.1.1.2 Figure 12 **Effects of PTmAb0011 on IgE secretion from primary human B-cells.**

20.1.1.3 Peripheral blood mononuclear cells ($2x10^5$/well) were cultured in medium supplemented with IL-4 (10ng/ml) and anti-CD40 antibody (1 μg/ml). PTmAb0011 or an isotype matched control mAb were added (1 μg/ml) for 14 days and then cell supernatant harvested and analysed for total IgE content by ELISA. The results are expressed as a percentage of the amount of IgE secreted in the absence of any antibody.

20.1.1.3.1.1.1 Figure 13A

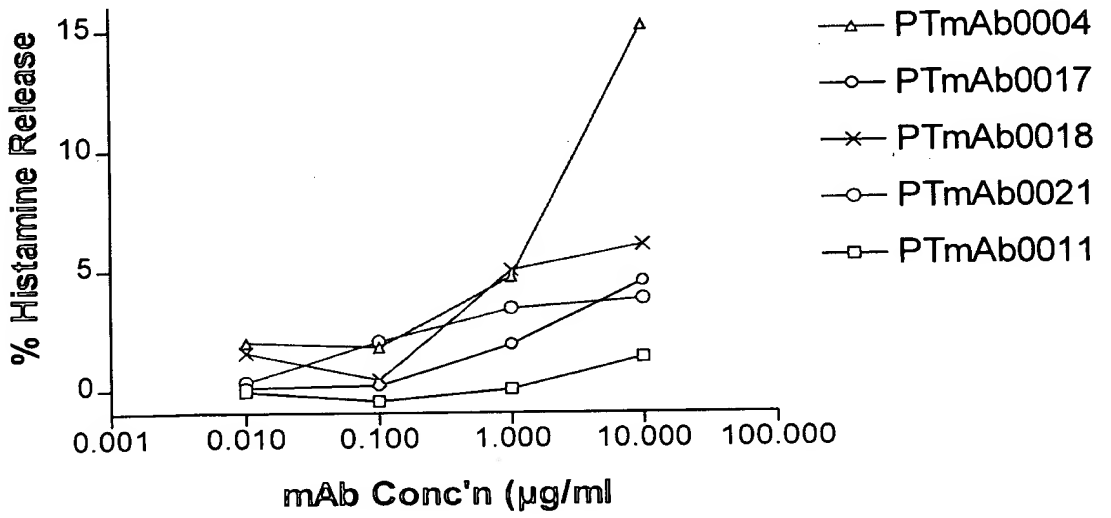


Figure 13B

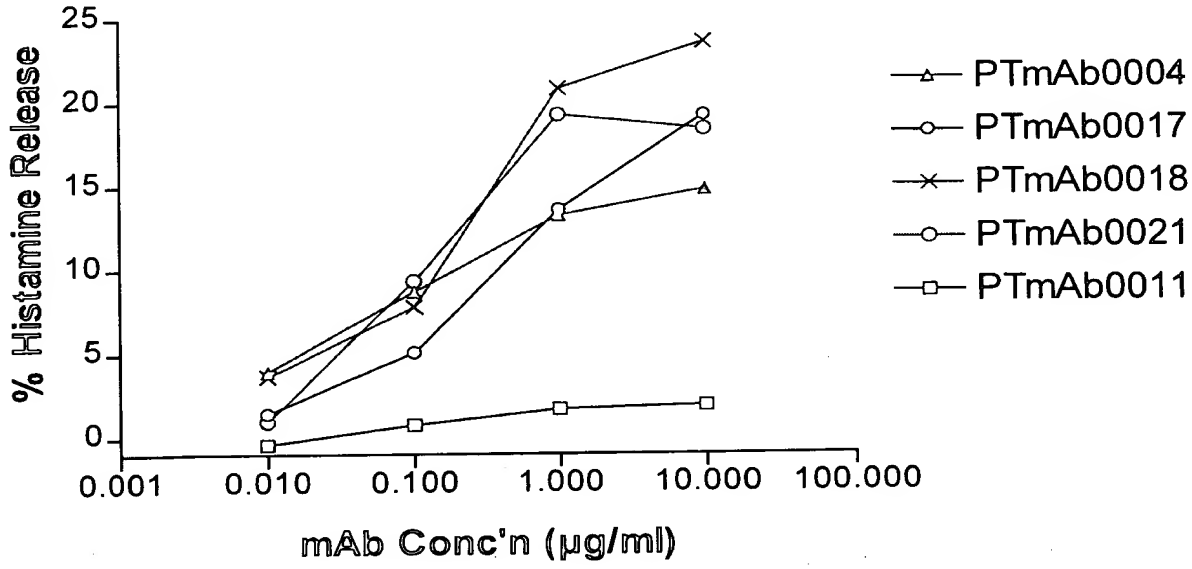
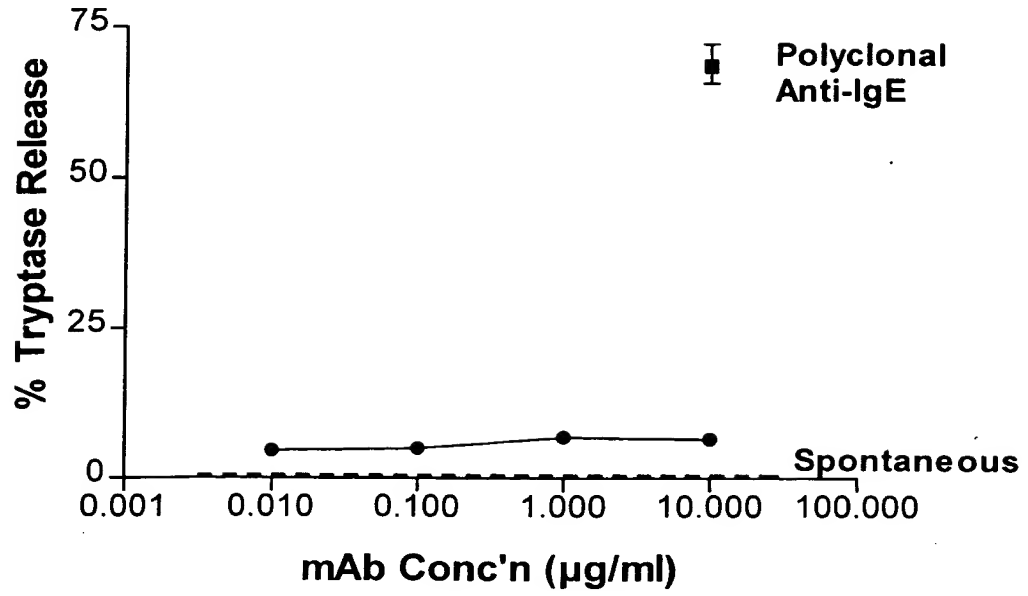


Figure 13 Anaphylactogenicity of anti-human IgE monoclonal antibodies in allergic (A) and non-allergic (B) human basophils

PBMC from allergic donors or from non-allergic donors passively sensitised with 1µg/ml chimeric IgE were treated with mAbs for 30 min. at 37°C. Histamine release was determined by specific EIA. Data are mean of 3 separate experiments each with different donors.

B45207
Figure 14A



20.1.1.3.1.1.1.1 ***Figure 14B***

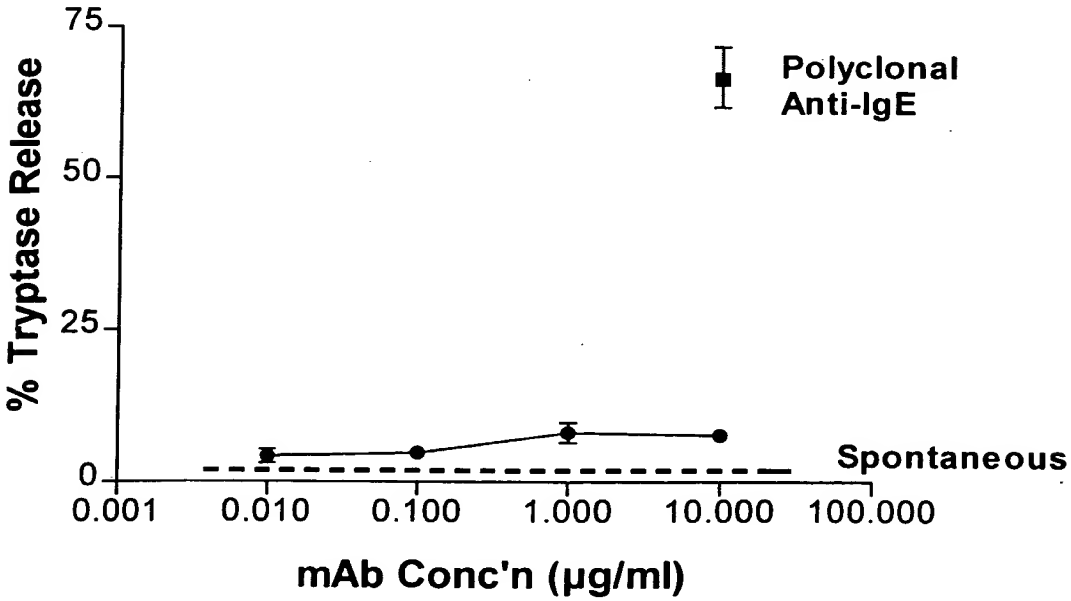
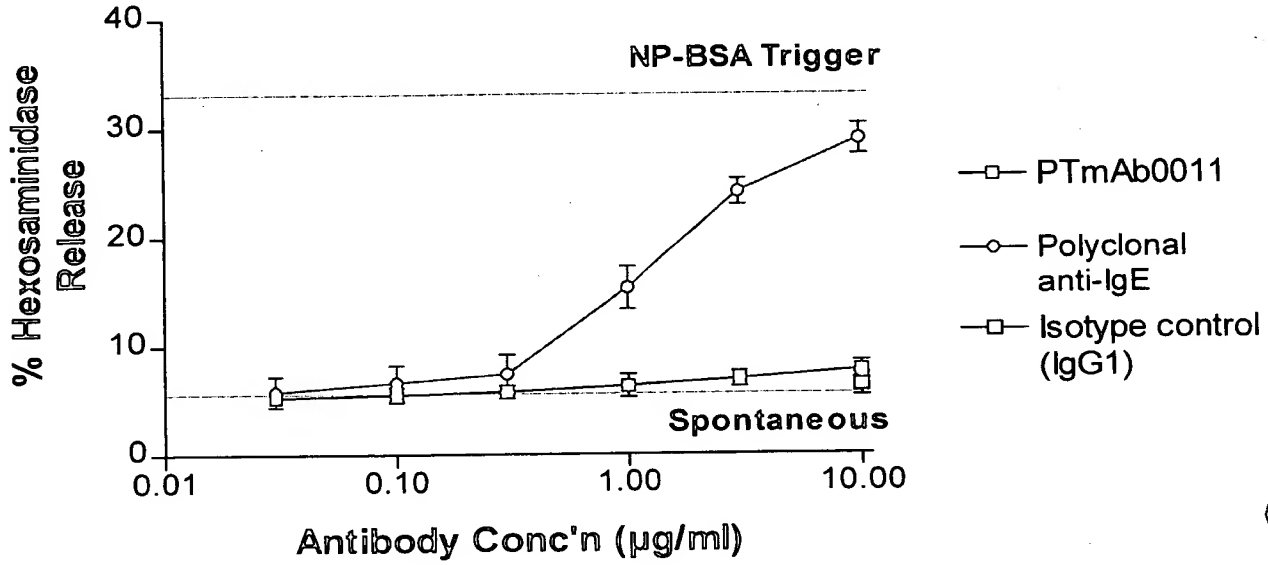


Figure 14 Anaphylactogenicity of anti-human IgE antibodies in sensitised (A) and non-sensitised (B) human lung mast cells

Sensitised or non-sensitised crude human lung mast cell suspensions were treated with antibodies for 45 min. at 37°C. Tryptase release in supernatants was determined by colorimetric assay. Data are means of duplicate determinations from a single representative experiment.

B45207
Figure 15A



20.1.1.3.1.1.1.1.1 **Figure 15B**

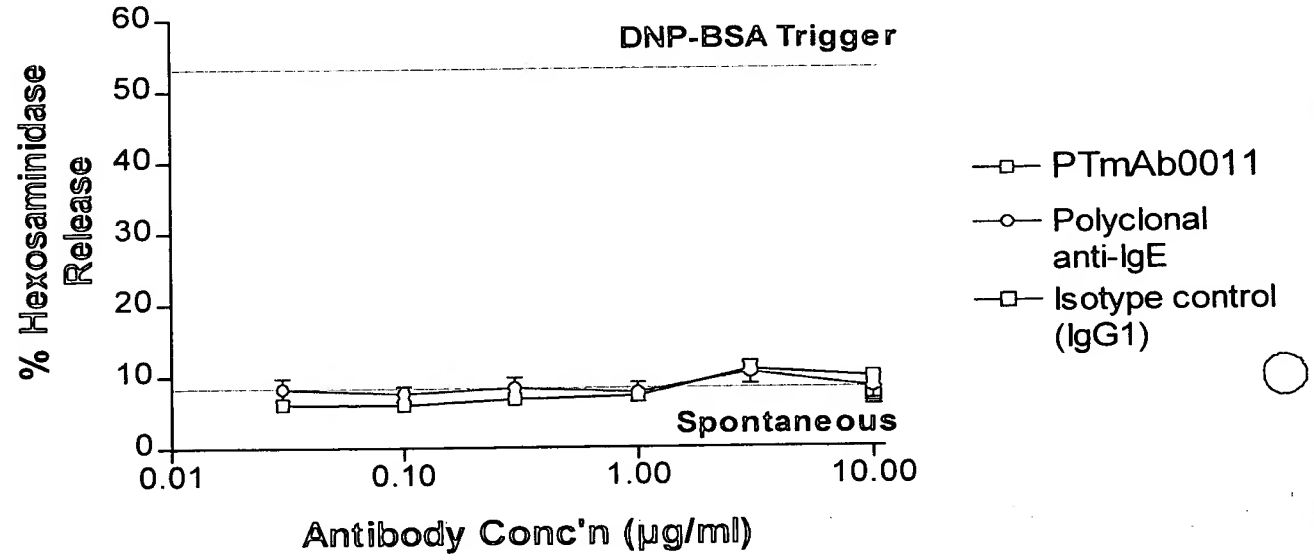


Figure 15 Anaphylactogenicity of anti-human IgE antibodies in RBL J41 cells through human FcεR1 (3A) and mouse FcεR1 (3B)

RBL J41 cells were sensitised either with chimeric human IgE or with mouse IgE and treated with antibodies for 30 min. at 37°C. β-hexosaminidase release was determined in supernatants by colorimetric assay. Data are means of triplicate determinations from a single representative experiment.